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Original Research Article

Association of Leptin and Insulin resistance among type 2 diabetes mellitus patients

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Abstract

Introduction- Diabetes is becoming more prevalent across the world. Type 2 diabetes is the most common type of diabetes and is characterized by hyperglycemia, insulin resistance, and insulin deficiency. Insulin secretion is compromised in these individuals and is inadequate to accommodate the insulin resistance in peripheral tissue. Hyperleptinemia reflects leptin resistance, which is a key factor in the production of IR in T2DM patients, making leptin a potential biomarker for evaluating IR levels.

Objective- This case-control study aimed to assess Leptin's association with insulin resistance among type 2 diabetes mellitus patients.

Material and methods- This case-control study was conducted in the MLB Medical College Jhansi. 73 patients diagnosed with T2DM and 40 healthy participants were enrolled according to inclusion criteria between 24-54 age groups and divided into three groups. In the Ist group, T2DM patients with metabolic syndrome enrolled according to WHO criteria (12). In the IInd group, T2DM patients were included without metabolic syndrome; in the IIIrd group, 40 healthy participants (male and female) were selected for control. Serum Leptin and insulin concentration were estimated using an ELISA kit method.

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Results- Leptin and Insulin levels were highly significant in patients with metabolic syndrome ($p<0.001$). The standard deviation of Serum leptin levels $10.01^{a}\pm2.7$, $6.9^{b}\pm2.4$ and $4.11^{c}\pm1.8$ and Serum insulin $120^{a}\pm40.7$, $20.43^{b}\pm5.2$, and $11.4^{b}\pm2.5$ in group I, 2 and 3 respectively were observed. There was a positive linear correlation between BMI, FBS, PPBS, HbA1c, TC, TG, Insulin and Leptin levels in the case group. An extremely significant correlation ($r=0.74$, $p<0.001$) was found in BMI and Leptin levels in the case group.

Conclusion- Serum leptin and insulin resistance syndrome have a positive association, with serum leptin being a significant predictor of insulin resistance syndrome. Leptin can be used as a metabolic syndrome diagnosis and early detection biomarker.

KEYWORDS: Leptin, Metabolic Syndrome, Diabetes Mellitus, Insulin

Introduction

Potentially, one of the oldest diseases in human history is diabetes mellitus. [1] In 1936, diabetes mellitus was classified as either type 1 or 2 [2]. In 1988, type 2 diabetes mellitus was initially identified as a part of the metabolic syndrome [3]. Type 2 diabetes, commonly referred to as non-insulin-dependent diabetes, is characterized by hyperglycemia, insulin resistance, and insulin insufficiency.[4] A combination of behavioral, environmental, and genetic risk factors leads to type 2 diabetes [5,6].

Globally, diabetes is becoming more widespread and common [7]. Over 250 million individuals worldwide have diabetes, and by 2030, that number is expected to increase to 400 million [5]. Hyperglycemia, a persistent rise in blood sugar levels brought on by either insulin secretion, insulin resistance, or both, is a metabolic disorder known as diabetes. Generally speaking, diabetes falls into two categories. [8]. First, there is type 1 diabetes, which affects five to ten percent of those with the disease. It is an autoimmune condition that causes pancreatic cells to be destroyed, leading to an absolute lack of insulin. Secondly, type 2 diabetes affects more than ninety percent of those with the disease and is caused by a composite of relative and absolute insulin deficiency rather than total insulin deficiency [8]. Because of this, these people's insulin secretion is impaired and insufficient to account for the insulin resistance in peripheral tissue [9]. Because it contributes to the inflammation of fatty tissue, the hormone leptin, which is generated from adipocytes, is also referred to as an adipocytokine [10]. It does a lot of things, the most crucial

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of which is to keep the energy balance [11]. This is achieved by increasing energy expenditure and decreasing appetite or energy consumption. It is, hence, appropriately called the satiety hormone [12]. Leptin has recently been found to regulate peripheral tissue sensitivity and insulin secretion [13]. Leptin is a promising biomarker for assessing IR levels since it is a major contributor to the creation of IR in T2DM patients due to hyperleptinemia, which reflects leptin resistance [14].

Moreover, it has been suggested that leptin levels, unaffected by obesity, can forecast the onset of metabolic syndrome [15]. Leptin affects hunger and obesity and raises blood pressure by stimulating the hypothalamic-sympathetic nervous system [16]. The elevated renal sympathetic tone observed in individuals who are overweight is thought to be caused by high amounts of circulating leptin [17]. Leptin is a known cause of hypertension, angiogenesis, and atherosclerosis [18,19]. These conditions can be detected early and used as biomarkers to diagnose metabolic syndrome.

While the exact cause of metabolic syndrome and its constituent parts remains unknown, two known contributing factors are central obesity and insulin resistance [20]. The diagnostic criteria for metabolic syndrome include obesity [waist circumference or BMI, TG (triglyceride) levels, hypertension, hyperglycemia, and urine albumin or albumin and creatinine ratio, according to numerous organizations. Regardless of the parameters employed, the main goal of too many diverse organizations is the early detection of potential CVD issues and their prompt action [21]. The current study aimed to assess the relationship between insulin and leptin and the metabolic syndrome.

Materials and Methods

This case-control study was done between February 2012 to June 2012 approved by the Institutional Ethical Committee.

Inclusion criteria: Forty healthy subjects between the ages of 24 and 53 were included, and 73 patients with T2DM were enrolled by ADA guidelines [22]. Each case and the control subject were divided into three groups. T2DM patients with metabolic syndrome were enrolled in the Ist group based on WHO guidelines [12]. Thirty male and female healthy individuals were chosen as

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controls in the third group, whereas T2DM patients without metabolic syndrome were included in the second group.

Exclusion criteria- Participants with other conditions, such as renal failure, liver cell failure, respiratory failure, or cardiac failure, were eliminated.

All patients were included following a thorough clinical examination and standard investigations, including lipid profiles, fasting and postprandial plasma glucose testing, complete blood counts, liver and kidney functioning tests, and anthropometric evaluations. Blood was drawn to measure insulin and leptin.

Biochemical evaluations: An insulin kit and a sandwich ELISA kit were used to determine leptin and insulin serum concentrations using the previously outlined methodology [19].

Anthropometric evaluations involved weight and height measurements. The weight was determined for the closest 0.1 kilograms. Height was measured with a measuring tape, and BMI was computed by dividing the weight in kilograms by the square of the height (m^2). Over 25 kg/m^2 was deemed obese by BMI. Dyslipidemia was diagnosed in patients with TC levels greater than 200 mg/dl, TG levels greater than 150 mg/dl, HDL-C levels less than 40 mg/dl in men and less than 50 mg/dl in women, and low-density lipoprotein cholesterol levels greater than 100 mg/dl.

Statistical Analysis- The Kolmogorov-Smirnov test was used to examine the normalcy of continuous data. The chi-square test was applied appropriately to compare the groups in non-normal continuous data. A one-way ANOVA was used for multiple group comparisons, with either the Tukey or LSD post hoc test coming next. A 2x3 contingency table calculator, accessible online at (http://faculty.vassar.edu/lowry/VassarStats.html), was used to compare gender data between groups. The statistical software for social science, version 22 (SPSS-22, IBM, Chicago, USA), was used for the statistical study. A two-tailed p-value of less than 0.05 is regarded as significant.

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Results

Table 1 shows the age ranges for groups 1, 2, and 3: 24-54, 26-51, and 25-53 years, respectively, with means of 43±7.15, 40.2±7.06, and 41.8±6.19 years. The post hoc test revealed that the groups' mean ages were similar ($p>0.05$), meaning they had no significant difference. Put otherwise, all the groups' subjects were of the same age. Furthermore, there was no significant difference ($p>0.05$) in the gender 2x3 chi-squire test between the chosen three groups. However, group 1's BMI was found to be considerably ($p<0.001$) greater than that of groups 2 and 3. Additionally, group 2 and group 3 exhibit homogeneity according to the post hoc ANOVA test ($p>0.05$).

Table 1: General Baseline characteristics of subjects

Variable	**Group1 (n=38)**	**Group2 (n=35)**	**Group3 (n=40)**	**F value**	***p-value***
Age					
(mean±SD)	43^{a}±7.15	40.2^{a} ±7.06	41.8^{a} ±6.19	1.52	2.14
Range	*24-54*	*26-51*	*25-53*		
Gender					
Male	22	16	23	Chi squire 1.54	1.52
Female	16	19	27		
BMI					
mean±SD	36.4^{a} ±4.85	18.3^{b} ±3.81	19.5^{b} ±4.07	211.13	<0.001
Range	22-43	12-24	17-30		

Age and BMI Significance were calculated by Post hoc test ANOVA Tukey test; for the post hoc test, the same alphabet shows homogeneity, and for gender 2x3 chi-square test was used at $p<0.05$.

Table 2 provides an overview of the groups' biochemical parameter levels. A post hoc LSD test comparing the three groups' mean biochemical parameter levels revealed a significant difference (all $p<0.01$) in the fasting, postprandial, and glycated blood sugar (FBS), as well as HbA1c levels among the chosen groups. Both groups had significantly different mean lipid profiles, with serum triglycerides (TG: mg/dl) and serum cholesterol (TC: mg/dl) being greater in group 1 than in the other two (both $p<0.001$).

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Table 2: Distribution of fasting blood sugar postprandial blood sugar, glycated haemoglobin (mg/dl) and lipid profiles between the test groups.

Parameters	**Group1 (n=38)**	**Group2 (n=35)**	**Group3 (n=40)**	**F(ANOVA)**
FBS (mg/dl)				
Mean± SD	155.4^{a}±27.1	103.7^{b} ±20.8	77.1^{c}±8.6	150.74
Range	*108-211*	*82-112*	*65-100*	
PPBS				
Mean± SD	288^{a}±58.8	193.9^{b}±18.5	108.3^{c}±12.1	238.3
Range	*210-390*	*170-225*	*85-119*	
HbA1C				
Mean± SD	11^{a}±1.41	6.88^{b}±1.07	4.28^{c}±1.05	315.17
Range	*7-11*	*6-11.5*	*3.8-6.2*	
TC (mg/dl)				
Mean±SD	250.7^{a} ±59.4	212.3^{b} ±38.8	177.5^{c} ±28.1	27.03
Range	*190-360*	*200-280*	*150-300*	
TG(mg/dl)				
Mean±SD	222^{a}±71.2	182^{b}±41.6	134.7^{c}±36.2	27.5
Range	*145-380*	*140-300*	90-220	

Significance was calculated by Post hoc test ANOVA LSD (Least significant difference) test; different alphabets in different parameters between groups shows significant variation at $p<0.01$.

Table 3 and Figure 1 demonstrated the mean serum leptin (ng/ml) and insulin (µIU/ml) in the studied groups. Comparing the distribution of leptin in studied groups, the Post hoc LSD test showed significant differences between all three groups ($p<0.001$). On the other hand, comparing the distribution of insulin in studied groups, statistical analysis showed a significantly different and higher frequency of high insulin group I compared to groups 2 and 3 ($p<0.001$) in Figure 2. In other words, type 2 diabetes may be related to Leptin and Insulin.

Table 3: Comparison between serum leptin and serum insulin of the studied groups

Parameters	**Group1 (n=38)**	**Group2 (n=35)**	**Group3 (n=40)**	**F (ANOVA)**

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Leptin (ng/ml)				
Mean±SD	10.01[a]±2.7	6.9[b] ±2.4	4.11[c]±1.8	65.2
Range	6-14	5-10	2-8	
Insulin (µIU/ml)				
Mean±SD	120 [a]±40.7	20.43[b] ±5.2	11.4[b]±2.5	243
Range	40-185	8-23	6-12	

Significance was calculated by Post hoc test ANOVA LSD (Least significant difference) test; different alphabets in different parameters between groups show significant variation at p<0.01.

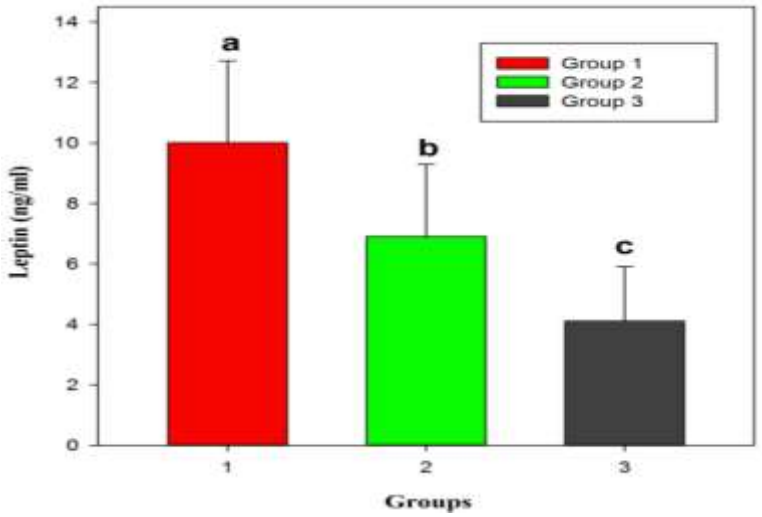

Fig. 1. Distribution of leptin in studied groups. Different alphabets in different parameters between groups show significant variation at p<0.001

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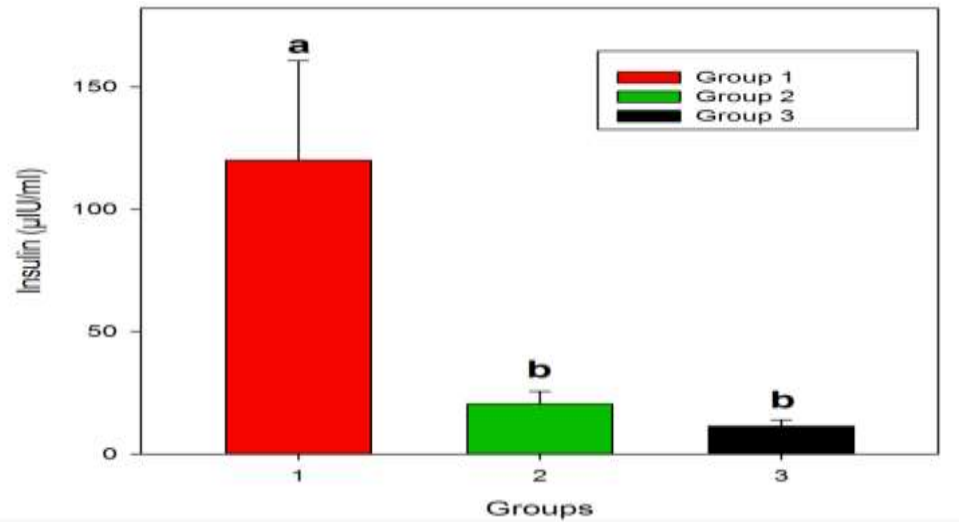

Fig. 2. Distribution of Insulin in studied groups. Different alphabets in different parameters between groups show significant variation at p<0.001

Relationship between baseline and biochemical parameters and serum leptin: In the case group (groups 1 and 2), there was a positive linear association between the selected parameters (BMI, FBS, PPBS, HbA1c, TC, TG, and Insulin) and the level of leptin (Table 4). The case group had a highly significant association (r=0.74, p<0.001) between the BMI and leptin level. Given its high value, leptin and BMI have a clear correlation. Age and serum leptin level were shown to have no connection (p>0.05).

Table 4: Correlation (r) between serum leptin and other parameters

Parameters	**r-*value***	***p-value***
Age	0.31	0.5
BMI	0.74	0.003*
FBS	0.68	0.002*
PPBS	0.41	0.02*
HbA1c	0.69	0.001*
TC	0.46	0.02*
TG	0.51	0.002*
INSULIN	0.54	0.003*

*significant p<0.05

Discussion

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The age and sex of the participants in this study showed statistically insignificant differences ($p>0.05$). However, when group 1's BMI was compared to groups 2 and 3, the disparities were noticeably greater ($p<0.001$). The results showed that the FBS levels in groups 1 and 2 were 155.4a±27.1 and 103.7b±20.8, respectively, while the PPBS levels in the control group were 108.3c±12.1, 193.9b±18.5, and 288a±58.8. These values were compared to 77.1c±8.6 in the healthy control participant. Compared to groups 2 and 3, the mean serum levels of these parameters were considerably higher in group 1 ($p<0.001$). The outcomes of the prior trial were the same [22]. Comparing HbA1c levels with those of patients without metabolic syndrome and healthy controls revealed that they were similarly statistically highly significant. Tamer et al. [19] have previously discovered statistical significance when comparing HbA1c to patients without metabolic syndrome and a healthy control group.

Additionally, TG and serum cholesterol levels were considerably higher in patients with metabolic syndrome healthy control than in patients without the condition ($P<0.001$). Furthermore, group 1 had considerably higher insulin and leptin levels ($p<0.001$) than groups 2 and 3.

The study demonstrated no significant differences ($p<0.001$) in the correlation between Leptin and the other parameters examined. However, there were significant differences ($p<0.001$) in the BMI, FBS, PPBS, HbA1c, TC, TG, and insulin. Our study's most significant finding consistent with the American Diabetes Association [22] was the positive correlation between serum leptin and serum insulin, or the idea that elevated serum leptin levels positively correlate with elevated insulin levels. As a result, we anticipate that elevated leptin levels will be present in insulin resistance syndrome patients experiencing a hyper-insulinemic state. Stated differently, we suggest leptin may be a useful marker for IR syndrome. Obesity increases the likelihood of leptin resistance, which in turn causes insulin resistance (IR), suggesting a role for leptin in the pathophysiology of type 2 diabetes [14, 23].

Welsh et al. [24] state that a man's leptin level can indicate his risk for type 2 diabetes. McNeely et al. found that elevated leptin levels were associated with an increased risk of type 2 diabetes [25]. These findings suggest a potential mechanism linking leptin levels in T2DM to insulin resistance. Moonishaa [14]. Our research shows serum insulin and leptin have a positive relationship with BMI. The positive association between serum insulin and leptin and BMI, as

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demonstrated by Moonishaa [14], implies that body fat or obesity contributes to developing hyperleptinemia and hyperinsulinemia in IR syndrome. Numerous research' findings indicate a high link between circulating leptin concentrations and obesity, despite leptin's anti-obesity capabilities [26,27]. Obesity and body fatness were found to correlate positively, with the obese population having very high serum levels of leptin [28]. This is further supported by Omar and Bahathiq's [29] findings, which showed a direct correlation between high levels of leptin and BMI and WC (waist circumference) in the non-diabetic and diabetic obese groups. In other earlier research, leptin and insulin were demonstrated to correlate positively with metabolic syndrome [30–34].

In contrast to earlier research, Martin et al. discovered a direct positive correlation between obesity and insulin resistance, hyperinsulinemia, and leptin, but not a significant correlation with the other elements of the metabolic syndrome [35, 36]. On the other hand, it has been determined that Leptin, an obesity marker, does not significantly contribute to the multifactor causation of interactions between adipocytokines that are thought to be involved in the IR defect. Furthermore, it is possible that leptin alone does not significantly correlate with the level of IR [34].

CONCLUSION

Our research led us to conclude that there is a positive correlation between serum leptin and insulin resistance syndrome. That serum leptin is a strong predictor of insulin resistance syndrome. Leptin is a biomarker that can diagnose and detect metabolic syndrome early on. The study's limited sample size is one of its limitations; therefore, we must investigate the impact of antidiabetic medication on leptin levels in a larger, longer sample.

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